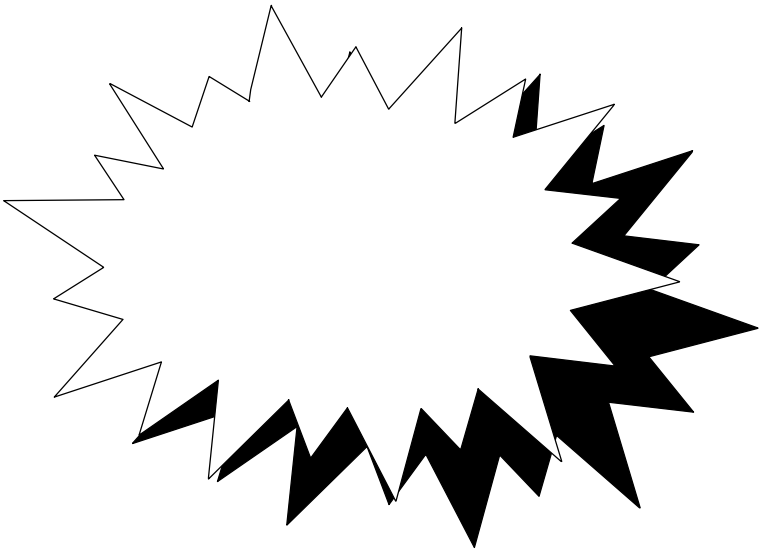

Chemical Analysis and Testing Task

Laboratory Analytical Procedure

LAP-004

Procedure Title: Determination of Acid-Soluble Lignin in Biomass

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Determination of Acid-Soluble Lignin in Biomass

Laboratory Analytical Procedure #004

1. Introduction

1.1 The residue remaining after extensive acid hydrolysis of a biomass sample, corrected for its ash content, is referred to as acid-insoluble lignin. This value, however, does not represent the total lignin content of the sample. A small portion of the lignin is solubilized during the hydrolysis procedure. This lignin fraction is referred to as acid-soluble lignin (ASL) and may be quantified by ultraviolet spectroscopy.

2. Scope

2.1 This procedure describes a spectrophotometric method for determining the amount of lignin solubilized upon hydrolysis of a biomass sample. The protocol utilizes the hydrolyzate generated by LAP-002, "Determination of Carbohydrates in Biomass by High Performance Liquid Chromatography", or by LAP-003, "Determination of Acid-Insoluble Lignin in Biomass".

2.2 Sample material suitable for this procedure include hard and soft woods, herbaceous materials (such as switchgrass and sericea), agricultural residues (such as corn stover, wheat straw, and bagasse), waste-paper (such as office waste, boxboard, and newsprint), washed acid- and alkaline-pretreated biomass, and the solid fraction of fermentation residues. All results are reported relative to the 105±C oven-dried weight of the sample. In the case of extracted materials, the results may also be reported on an extractives-free basis.

2.3 Liquid process samples may also be analyzed by this technique to give an estimate of their acid-soluble lignin content. The values generated must be viewed as estimates only since many other components present in liquid process samples will also absorb at the analysis wavelength and will bias the results high.

2.4 All analyses shall be preformed according to the guidelines established in the Ethanol Project Quality Assurance Plan (QAP).

3. References

3.1 Ethanol Project Laboratory Analytical Procedure #002, "Determination of Carbohydrates in Biomass by High Performance Liquid Chromatography".

3.2 Ethanol Project Laboratory Analytical Procedure #003, "Determination of Acid-Insoluble Lignin in Biomass".

3.3 Ethanol Project Laboratory Analytical Procedure #010, "Determination of Extractives in Biomass".

3.4 Kaar, W.E., and D.L. Brink. 1991. "Simplified Analysis of Acid Soluble Lignin." *Journal of Wood Chemistry and Technology*, 11(4):465-477.

3.5 TAPPI Test Method T250, "Acid-Soluble Lignin in Wood and Pulp." *In Tappi Test Methods.* Atlanta, GA: Technical Association of the Pulp and Paper Industry.

4. Significance and Use

4.1 The acid-soluble lignin determination is used in conjunction with other assays to determine the total composition of biomass samples.

5. Interferences

5.1 Any component besides acid-soluble lignin which is present in the hydrolyzate and which absorbs at the analytical wavelength of 205 nm will cause the results to be biased high. This problem will be most severe with liquid process samples which have been shown to contain components which absorb at 205 nm.

6. Apparatus

6.1 Spectrophotometer, suitable for measuring absorbance at 205 nm.

7. Reagents and Materials

7.1 Sulfuric acid, 4% w/w, prepared by diluting 3.00 ± 0.01 mL of 72% w/w H_2SO_4 with 84.00 ± 0.04 mL of deionized water.

> Note: For hydrolyzates generated from solid biomass samples taken through LAP-002 or LAP-003, the sulfuric acid used to prepare the 4% acid solution must be from the same prepared batch of 72% w/w H_2SO_4 used to prepare the sample.

7.2 Water, 18 megohm deionized.

7.3 Matched pair of quartz cuvettes with a 1-cm path length.

7.4 Glass test tubes, of a size suitable for making dilutions.

7.5 Adjustable pipettors of various sizes with the appropriate disposable tips.

8. ES&H Considerations and Hazards

8.1 72% H_2SO_4 is very corrosive and must be handled carefully.

8.2 Follow all applicable NREL Laboratory Specific Hygiene Plan guidelines.

9. Procedure

9.1 Set up and calibrate the spectrophotometer following the protocols recommended in the instrument manual.

9.2 Measure the absorbance of the hydrolyzate at 205 nm, using the 1-cm light path cuvette. A 4% solution of H_2SO_4 should be used as a reference blank.

9.3 If the absorbance reading exceeds 0.7, the sample must be diluted. Dilute the sample so the resulting absorbance reading falls between 0.2 and 0.7. The 4% H_2SO_4 must be diluted in the same ratio as the sample and used as the reference blank for this repeat analysis.

9.4 Repeat the analysis on a second aliquot of the hydrolyzate. Each sample must be analyzed in duplicate, including the hydrolyzates generated from the analysis of the LAP-002 or LAP-003 method verification standard.

10. Calculations

10.1 An absorptivity (extinction coefficient) value of 110 L/g-cm is used to calculate the amount of acid-soluble lignin present in the hydrolyzate. The 205 nm absorptivities reported for most woods fall in the range of 88 to 113 L/g-cm. The value of 110 L/g-cm used in this protocol is consistent with the value used in the TAPPI procedure and represents an average of values found for different woods and pulps.

> Note: An absorptivity for a given type of biomass may be determined using the "reKlasonation" protocol described in Kaar and Brink (1991).

10.2 For a liquid process sample, an estimate can be made of the amount of acid-soluble lignin present as follows:

$$ASL, estimated\ (g/L) = \frac{A}{b\ x\ a}\ x\ df$$

Where:

A= absorbance at 205 nm.
df = dilution factor.
b = cell path length, 1 cm.
a = absorptivity, equal to 110 L/g-cm unless experimentally determined for a given biomass material.

10.3 For a solid biomass sample, the percent acid soluble lignin on a 105°C dry weight or on an extractives free basis is calculated as follows:

$$\%\ ASL = \frac{\frac{A}{b\ x\ a}\ x\ df\ x\ V\ x\ \frac{L}{1000\ mL}}{\frac{W\ x\ T_{final}}{100}}\ x\ 100$$

Where:

A = absorbance at 205 nm.
df = dilution factor.
b = cell path length, 1 cm.
a = absorptivity, equal to 110 L/g-cm unless experimentally determined for a given biomass material.
V = filtrate volume, this volume will either be 87 mL, if the sample is the hydrolyzate from the carbohydrate analysis (LAP-002) or the LAP-003 summative acid-insoluble lignin protocol, or will be equal to the weight of the filtrate obtained in the LAP-003 Klason lignin protocol expressed in mL.
W = initial biomass sample weight in grams (from LAP-002 or LAP-003).
$\%T_{final}$ = % total solids content of the biomass sample (as received or after extraction), on a 105°C dry weight basis, as determined during the LAP-002 or LAP-003 analysis.

10.4 For an extracted biomass sample, the percent acid-soluble lignin value, calculated above, can be converted to an as received (whole sample) 105°C dry weight basis as follows:

$$\%\ ASL_{whole\ sample} = \%\ ASL_{extractives\text{-}free}\ x\ \frac{(100\% - \%\ extractives)}{100\%}$$

Where: % $ASL_{extractives\text{-}free}$ = % acid-soluble lignin on an extractives-free 105°C dry weight basis, as determined in the previous step.
% extractives = % extractives in the extracted sample as described in LAP-010, "Standard Method for the Determination of Extractives in Biomass".

11. Report

11.1 For solid biomass samples, report the percent acid-soluble lignin present in the sample, to two decimal places, on a whole sample 105°C dry weight basis or on an extractives-free basis. For liquid process samples, report the acid-soluble lignin content as an estimate, to two decimal places. Cite the basis used in the report.

11.2 For replicate analyses of the same sample, report the average, standard deviation, and relative percent difference (RPD).

12. Precision

12.1 Data obtained by replicate testing of a hybrid poplar in one laboratory gave a standard deviation of 0.12% and a CV of 5.41%.

12.2 Data obtained by replicate testing of a hybrid poplar sample in six different laboratories gave a standard deviation of 0.98% and a CV of 41%.

13. Quality Control

13.1 *Reported significant figures:* For solid biomass samples, report the percent acid-soluble lignin present in the sample, to two decimal places, on a whole sample 105°C dry weight basis or on an extractives-free basis. For liquid process samples, report the acid-soluble lignin content as an estimate, to two decimal places. Cite the basis used in the report.

13.2 *Replicates:* At minimum, all samples and the method verification standard are to be analyzed in duplicate.

13.3 *Blank:* Dilute the 4% sulfuric acid solution in the same manner as the sample and use as the reference blank in the spectrophotometer.

13.4 *Relative percent difference criteria:* The RPD must be less than 15.5%. If the RPD is too large, the sample must rerun.

13.5 *Method verification standard:* A method verification standard must be run in duplicate with every batch. This method utilizes the hydrolyzate generated by LAP-002 or LAP-003 from a well characterized standard material suitable for analysis. For example, NIST 8492 (*Populus deltoides*) is used as the MVS in compositional analysis of hardwoods.

13.6 *Calibration verification standard:* Not applicable.

13.7 *Sample size:* A minimum of 5 mL of hydrolyzate generated by LAP-002 or LAP-003 are required for duplicate analyses. If there is insufficient sample, the result will be flagged and the lack of precision will be noted.

13.8 *Sample storage:* Samples shall be stored in an airtight container and refrigerated. They must be analyzed within 24 hours, and preferably within 6 hours, of the hydrolysis step of LAP-002 or LAP-003.

13.9 *Standard storage:* Not applicable.

13.10 *Standard preparation:* Not applicable.

13.11 *Definition of a batch:* Any number of samples which are analyzed and recorded together. The maximum size of a batch would be limited by the equipment constraints. A batch cannot be larger than what is practical with the equipment.

13.12 *Control charts:* The result of each replicate analysis of the method verification standard is recorded along with the average, RPD, and a laboratory book/page reference. The average value obtained for each analysis of the method verification standards is to be control charted.